

## ABSTRACTS OF PAPERS

### Twelfth Annual Albert L. Tester Memorial Symposium 2-3 April 1987<sup>1</sup>

The Albert L. Tester Memorial Symposium is held in honor of Professor Albert L. Tester who, at the time of his death in 1974, was Senior Professor of Zoology at the University of Hawaii. The faculty and students of the Department of Zoology proposed an annual symposium of student research papers as a means of honoring, in a continuing and active way, Dr. Tester's lively encouragement of student research in a broad range of fields in marine biology. Papers reporting original research on any aspect of biology are solicited from students at the university and these papers are presented at the symposium, which takes place during the spring semester. Income from contributions to the Albert L. Tester Memorial Fund of the University of Hawaii Foundation is used to provide two prizes for the best paper by graduate students. Papers are judged on quality, originality, and importance of research reported, as well as the quality of the public presentation. Judges include several members of the faculty of the Department of Zoology as well as winners of the symposium from the preceding year, when possible. In addition, a distinguished scholar from another university is invited to participate in the symposium as a judge and to present the major symposium address. This year Lynn Margulis of Boston University participated in the symposium.

### Effects of Diet on Growth, Metamorphosis, and Survival of Gastropod Larvae

JANICE BELL<sup>2</sup>

For marine invertebrate larvae, both growth rates and residence time in the plankton can affect mortality in the plankton, timing of metamorphosis, proximity to recruitment sites, and success of recruitment. These factors in turn may be used to predict or explain success in terms of abundance, geographic distribution, or longevity in the fossil record. Actual growth rates of larvae in the plankton are difficult to determine. Thus, rates of growth resulting from laboratory culture are generally considered the best estimates of larval growth. The prosobranch gastropod, *Crucibulum spinosum*, was chosen to compare growth rates in static laboratory cultures and those in natural seawater. Ingestion rates, as determined by particle counts, were com-

pared for various phytoplankton species. Larvae were fed monocultured phytoplankton in various combinations with a total density of  $1 \times 10^4$  cells per ml in static cultures. Species included Tahitian *Isochrysis galbana*, *Phaeodactylum tricornutum*, *Chaetoceras gracilis*, and a local flagellate. Larvae were also cultured in drip-thru baskets on a seawater table. In static culture, larvae grew fastest on a combination of Tahitian *I. galbana*, *P. tricornutum*, and the local flagellate. Metamorphosis occurred 23 days after hatching but survival was low. Survival was higher without the local flagellate, but larvae did not metamorphose until 29 days after hatching. However, in the drip-through seawater system, larvae grew fastest, reaching metamorphosis in 16 days. Metamorphosis was not induced by any specific agent or treatment. Metamorphic size ranged from 730-

<sup>1</sup> Manuscript accepted May 1987.

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840  $\mu\text{m}$ . This rapid growth appears to be a rare event. Subsequent experiments compared larval growth between drip-through seawater and enhanced drip-through in which monocultured phytoplankton supplemented the drip-through seawater. Larvae from the same egg mass grew in the phytoplankton-enhanced drip-through system and showed no growth in the seawater drip-through system. Using larvae from the same egg mass

in both feeding systems demonstrated that the larvae that did not grow were not deficient or damaged. Particle counts showed coastal seawater to have  $3\text{--}5 \times 10^3$  particles per ml in size ranges captured by larvae. Possibly a bloom of a phytoplankton species important to larval growth causes an occasional rapid growth rate. This may be a rare event in nature.

### Effects of Digestive Grazing by the Sea Star *Patirella regularis* on Communities of Coralline Algae<sup>3</sup>

JEFF M. BURGETT<sup>4</sup>

The asterinid sea star *Patirella regularis* occurs in intertidal zones of New Zealand dominated by coralline algae, which are resistant to most herbivores but are visibly scarred by *P. regularis*. Like other grazing asteroid, *P. regularis* digests its prey *in situ*, and therefore may be able to circumvent some structural features which defend benthic algae against dentate herbivores. Surveys showed that *P. regularis* was positively associated with coralline crusts and was most often found feeding on these algae. Turfs of *Corallina officinalis* were apparently avoided, although scanning electron microscopy indicated that turfs as well as crusts of coralline algae were vulnerable to digestive attack by *P. regularis*. A factorial caging experiment was conducted over 144 days to investigate the effects of three levels of asteroid grazing

on primary succession and on communities dominated by perennial coralline crusts and turf. Control treatments allowed estimation of shading and gastropod exclusion effects. Densities of *P. regularis* 8 and 16 times above ambient reduced plant diversity, lowered the abundance of coralline algae, and suppressed colonization of open space by non-encrusting algae. Twice the observed natural density of *P. regularis* caused no significant changes in the established or developing algal communities. These results suggest that intertidal populations of *P. regularis* have little direct effect on algal community structures at natural densities. However, by differentially affecting coralline crusts, low densities of this asteroid could alter competitive relationships among encrusting algae.

### Plasmid Control of Symbiotic Properties in *Rhizobium fredii*<sup>5</sup>

MARIA LUZ F. CACES<sup>6</sup>

*Rhizobium fredii*, a fast-growing rhizobium that nodulates soybeans, was used to

study the involvement of plasmids in the regulation of the  $\text{N}_2$ -fixing symbiosis. Five strains of *R. fredii* (USDA 205, HH 003, HH 102, HH 103, and HH 303) were examined for the presence of indigenous plasmids. To determine if symbiotic functions are controlled by genes on the plasmids, the strains were subjected to plasmid-curing treatments. The effect of a pSym plasmid from a heterologous species on *R. fredii* gene function was

<sup>3</sup>This study was partly funded by a fellowship from Rotary International.

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studied by the introduction of the *R. leguminosarum* pSym plasmid, pJB5JI.

The results showed that high molecular weight plasmids that are involved in determining symbiotic functions, as well as cryptic plasmids, are an integral part of the genetic makeup of *R. fredii*. In two of the strains, the loss of the ability to nodulate soybeans was correlated with the loss of a plasmid.

The strains received and maintained pJB5JI but the pJB5JI genes were not expressed in the *R. fredii* genetic background. The introduction of the plasmid did not en-

able any of the *R. fredii* transconjugants to nodulate peas, nor did it restore the ability of the plasmid-cured transconjugants to nodulate soybeans. The *R. fredii* symbiotic genes were variably affected by the heterologous plasmid, with the transconjugants exhibiting different levels of symbiotic effectiveness that ranged from ineffective nodulation to N<sub>2</sub>-fixation levels comparable to those of the parental types. *R. fredii* gene expression in the presence of pJB5JI was unstable and changed with successive passage on the host plant.

## The Biochemical Basis of Cold Tolerance in *Aphelenchus avenae* (Nematoda)<sup>7</sup>

DAVID W. DEXTER<sup>8</sup>

Many nematodes, particularly free-living and plant-parasitic forms, have been shown to have remarkable capacities for dehydration survival. Their survival correlates with increased concentrations of certain low molecular weight carbohydrates, particularly glycerol and trehalose, which are important to the maintenance of functional integrity during water removal. However, many of the organisms studied thus far are temperate species existing in an environment prone to cold stress and freezing rather than dehydration. This research has been conducted to test the hypotheses that (1) because these organisms live in temperate and not arid climates they should be adapted to survive cold stress and (2) cold survival is facilitated by the production of low molecular weight carbohydrates which secondarily allow these temperate forms to survive dehydration.

The temperate, free-living, mycophagous nematode *Aphelenchus avenae* was chosen because of its abilities to survive dehydration. Samples were maintained for 24, 48, and 72 hr at 0.5–1.5°C and subsequently immersed in liquid nitrogen and lyophilized. Survival rates were compared with samples

maintained at room temperature and samples which were frozen just below 0°C after 72 hr at 0.5–1.5°C. Other nematode samples were frozen at –5°C directly after extraction from culture and after 72 hr at 0.5–1.5°C. Carbohydrates were isolated using a chloroform/methanol extraction technique. Lyophilized samples were homogenized in chloroform and methanol. Carbohydrates, contained in the methanol layer, were concentrated to dryness by rotary evaporation. Proteins were precipitated out in ethanol. Carbohydrates were identified and quantified by High Performance Liquid Chromatography (HPLC), and compared with observed responses of known standards.

Revival rates of samples incubated at 0.5°C were almost 100%. Samples frozen slowly at temperatures slightly below zero after the 72 hr “preparatory period” had revival rates greater than 80%. *A. avenae* was unable to survive slow freezing without a preparatory period or rapid freezing with a preparatory period. During the 72 hr preparatory period the concentrations of glycerol, trehalose, glucose, and a presently unidentified carbohydrate (thought to be a trisaccharide) remained relatively constant. Subsequent exposure to subfreezing temperatures caused drastic increases in the concentrations of glucose, glycerol, trehalose, and the unidentified carbohydrate, with trehalose concentrations

<sup>7</sup>This research was funded by a grant from the U.S. Department of Agriculture, grant no. F-87-222-F-542-B-716, awarded to Dr. Christopher Womersley.

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being the highest. In addition, the concentration of myo-inositol, which appeared in small concentrations during the third day of incubation, increased dramatically as a result of freezing stress.

Increases in carbohydrate concentrations caused by exposure to subfreezing temperatures and the resulting high survival rates support the hypothesis that temperate nema-

tode species are adapted to tolerate freezing stress. Based on the conditions these organisms must endure in temperate winters, it is suggested that low molecular weight carbohydrates are produced primarily as a mechanism for survival at reduced, and ultimately, freezing temperatures and secondarily as a mechanism to survive dehydration.

## A Characterization of Polychaete Assemblages on a Hawaiian Fringing Reef<sup>9</sup>

MARGARET DUTCH<sup>10</sup>

In an effort to understand better the composition of cryptofaunal and infaunal invertebrate communities of tropical reef flats, a survey was undertaken to compare the polychaete assemblages in carbonate sand, rubble, and reef rock. Ten replicate samples of each substratum type were taken from a location on the Kahala reef flat approximately 80 m from shore at a depth of 1 m. All 30 samples were taken within an area 3 m in radius to ensure equal exposure to physical environmental conditions. Samples were preserved and washed to recover organisms not firmly fixed in tubes and burrows. Tube- and burrow-dwelling organisms were recovered by placing samples in a nitric acid bath to dissolve calcium carbonate. All polychaetes recovered are being identified, enumerated, and placed in feeding and locomotory categories. To date, 12,130 individuals have been identified

and placed into 44 species. Analysis of these data indicates that all three substrata differ in (1) the total number of individuals and species per replicate, (2) the composition of the polychaete assemblages, and (3) the various feeding and locomotory adaptations displayed by the polychaetes. Thus, the polychaete assemblages found in sand, rubble, and rock substrata differ significantly in the parameters examined. Although it was anticipated that differences would be found between soft (sand) and hard (rubble and rock) substrata, the dissimilarity seen between the polychaete assemblages of the rubble and rock substrata was less expected. Further examination of the distribution of species among the substrata and of the physical characteristics of the substrata (e.g., grain size and porosity) should be useful for interpreting these results.

## Differential Growth and Survival of Juvenile Hawaiian Corals<sup>11</sup>

R. C. FITZHARDINGE<sup>12</sup>

<sup>9</sup>This research was supported in part by the International Women's Fishing Association Scholarship Trust.

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<sup>11</sup>The research was supported by a grant the University of Hawaii Sea Grant College Program to Dr. J. Bailey-Brock.

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The influence of fish-grazing on the recruitment of three common Hawaiian corals, *Pocillopora damicornis*, *Porites compressa*, and *Montipora verrucosa*, was investigated using concrete blocks that were uncaged, caged, or placed under roofs. The experiment provided an opportunity to compare the growth rates and survival of these species. The blocks were placed in the field



during June 1986 and examined in September of the same year. Reproduction of all three species occurs during these three months. Corals on the sides of blocks were counted, and the number of polyps and maximum diameter of colonies were recorded. Caged and uncaged blocks were replaced and re-examined five months later in February 1987. The highest recruitment of all three species occurred on the caged blocks that were examined in September. *Montipora verrucosa* was the most common recruit with densities of up to 39 colonies/100 cm<sup>2</sup> on caged blocks. At both samplings, *P. damicornis* recruits were larger than *P. compressa* and

*M. verrucosa*, based on the mean maximum diameter of colonies or on the number of polyps per colony. The results support the hypothesis that fish-grazing negatively impacts newly settled corals. Newly settled *M. verrucosa* polyps are probably most susceptible to grazing because they are small in size and are apparently slower growing than *P. damicornis* or *P. compressa*. Thus, high postsettlement mortality, not low settlement rates, probably explains the low recruitment rates previously reported for this species. The success of *P. damicornis* in colonizing new substrata also may be linked to high juvenile growth rates in this species.

### **The Effect of Protein Level in Isocaloric Feeds on the Growth Performance of *Macrobrachium rosenbergii* Individually Reared in Clear Water Flow-Through Aquaria<sup>13</sup>**

GEORGE W. FRUECHTENICHT<sup>14</sup>

Knowledge of the nutrient requirements for any cultured species is critical for optimization of growth and efficiency of feed formulation. In order to determine nutrient requirements of a social aquatic detritivore such as *Macrobrachium rosenbergii*, experimental trials need to be conducted in clear water aquaria to avoid the nutritional contribution of natural foods. Also, since social interaction plays a significant part in food acquisition in this species, individual housing is recommended for nutrition trials.

Two lab trials were conducted with several size groups of *M. rosenbergii* to study the effects of dietary protein levels on the growth performance of prawns. In the first trial, 25 individually housed prawns of three size groups ( $\bar{x}$  = 2.2 g, 10.8 g, and 22.5 g) were fed one of three isocaloric feed formulations

which varied in protein level (14.1, 30.3, and 51.2%). The prawns were acclimated to the aquaria and respective feed formulations for one week; individuals were then weighed and measured and grown over a twelve-week period with measurements taken every four weeks. The prawns were fed once daily in the afternoon, and the amount of feed applied and quantitative acceptance of the feed were noted. Significant differences were obtained; animals on the 14.1% protein diet performed less well than those on the higher protein groups for all size classes of prawns. There were no differences in growth on the 30.3 and 51.2% protein diets. In the second trial, two size classes of prawns (3.1 g and 13.8 g) were given one of three isocaloric feed formulations varying in protein level within a narrower range (15.3, 23.6, and 32.3%). The experimental procedure was the same as trial one. In the large prawn size class, growth increased significantly with increasing protein level up to 32%. In the small prawn size class, the high and mid-protein groups grew equally well, but outperformed the low protein group. These data suggest that both

<sup>13</sup>Funding for materials for this experiment was provided by the University of Hawaii Sea Grant College Program and the Aquaculture Research Program, Department of Animal Sciences, University of Hawaii.

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juvenile and adult freshwater prawns may require a dietary protein level of around 30%, while smaller prawns may have an overall lower protein requirement of 23–25%.

## Nervous System Development Studies in Tobacco Hornworm Heterochronic Chimeras<sup>15</sup>

ROBERT A. GRAF<sup>16</sup>

Hormonal cues dictate the development of the nervous system in the larval form of the tobacco hornworm, *Manduca sexta*. During the larval to pupal transformation a sequence of hormonal events results in normal development. Specifically, the blood titer of juvenile hormone (JH) decreases, followed by a small ecdysone (E) peak in the absence of JH called the "commitment pulse." Once this pulse occurs, the tissue is committed to undergo further development. However, if cells are treated locally with JH so that it is present at the time of the commitment pulse, the effects of the pulse are masked and the treated tissue

is "fixated" in development until a subsequent E pulse occurs. The hormonal manipulation results in a heterochronic chimera. The time over which this chimera can be induced defines the critical "window."

Centrally located motor neuron somata and peripherally located sensory neurons form a reflex arc (Weeks and Jacobs 1986, *J. Comp. Physiol. A*, in press). Ectopic treatment of proleg sensory neurons with JH preferentially fixates these cells. The critical window for fixation was determined to be from 3–7 P.M. of larval day 2.

## Age in a New Light: Gerontological Studies of the Damsel fish, *Dascyllus albisella*

KEVIN HILL<sup>17</sup>

Most studies concerning the growth of fish have dealt with the estimation of chronological age, usually determined by: length frequencies, analyses of rhythmic patterns found in calcified structures, and laboratory growth experiments. It is now possible to also define physiological age through assays of the gerontological, metabolically accumulated cellular pigment, lipofuscin. The purpose of the

present research was to examine lipofuscin as a means of defining "physiological" age in the damselfish, *Dascyllus albisella*, in order to compare chronological age with physiological age. Lipofuscin pigments were extracted from brain tissues using a chloroform/methanol solvent system. Chloroform soluble pigments were then detected by fluorescence spectrophotometry. Chloroform lipofuscin extracts had clearly defined excitation and emission spectra in all samples with no evidence of interfering products. Chronological age was estimated through the utilization of rhythmic patterns in sagittal otoliths which were analyzed by light and scanning electron microscopy. Assays of chloroform-

<sup>15</sup>This research was supported by an NIH grant to Dr. J. C. Weeks of the Department of Entomology, University of California at Berkeley.

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extractable pigments demonstrated that lipofuscin concentrations in brain tissues were in direct relationship to both fish size and chronological age. These data provide evidence for the presence and accumulation of lipofuscin pigments in the brain cells of

*D. albisella*, and suggest a relationship to physiological aging processes. Studies of lipofuscin accumulation in fishes hold great potential for answering new questions regarding fish aging processes, longevity and general ecology.

## **Genotypic Diversity and Clonal Fitness of the Reef Coral *Porites compressa* from Three Populations with Different Disturbance Histories**

C. L. HUNTER<sup>18</sup>

Genotypic diversity in populations of clonal organisms is a function of the relative contributions of sexual and asexual reproduction to the population as a whole; young (or recently disturbed), sexually recruited populations will have a higher genotypic diversity than older (or more physically stable) populations in which vegetative propagation of locally fit genotypes has led to numeric and spatial dominance of a few clonal types. I tested this hypothesis for three populations of the endemic Hawaiian coral *Porites compressa*. Clonal dispersion and genotypic diversity were measured by sampling five individuals from each of nine nested quadrats in each population. Six enzyme loci were assayed for each of the 45 individuals per population. Genotypic diversity, calculated as  $G_0$ , was highest in the population which

was intermediate in disturbance history, and lower in both the temporally younger (extremely disturbed) and older (relatively undisturbed) populations. Common garden (reciprocal transplant) experiments showed that numerically abundant clonal types grew faster than rare clones, but that rare clones may be more competitively aggressive in inter-clonal contacts. Asexual fecundity (the relative ability to produce viable vegetative propagules) and the survivorship of fragments from rare and common genotypes are currently being measured. Results of these studies indicate that evolutionary models based on simple Mendelian population genetics may not be generally applicable to the large and ecologically important groups of organisms which employ asexual reproduction as part of their life histories.

## **Timing of Larval Release and Settlement in Sessile Marine Invertebrates<sup>19</sup>**

CATE HURLBUT<sup>20</sup>

Larvae of sessile marine invertebrates are at the mercy of planktonic conditions which

must be survived for larvae to settle and subsequently reproduce. Evolutionarily, two ways which adults can attempt to increase chances of larval survival are by altering larval characteristics (e.g., swimming ability, planktonic period), or by changing the time when larvae are released into the plankton. The majority of larval studies have focused

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<sup>19</sup>This research was funded by a Sigma Xi Grant-in-Aid and the Department of Zoology, University of Hawaii.

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on the first category. I examined patterns of larval release in the field by documenting settlement of several sessile marine invertebrates on diurnal and seasonal scales. Organisms with short-lived or long-lived larvae were compared so that the consequences of different life history strategies of adults on fitness may be examined in future studies.

I used acrylic plates immersed for varying periods (one hour to one week) to document settling times of fouling organisms in Pearl Harbor, Oahu. Abundant settlers included serpulid and spirorbid polychaetes, the bivalve *Anomia nobilis*, the bryozoan *Schizoporella unicornis*, and several species of tunicate.

Weekly settlement rates were examined from June to December 1986. In general, settlement was highest during late July and early August, although there were several patterns of peak settling times. Serpulids and *S. unicornis* settled in high numbers during early summer and had a peak in August. Spirorbrids had low settlement during summer and fall and a sharp increase during December. *A. nobilis* had the most restricted settlement period, settling mostly during July and August. Tunicate species all showed similar

patterns, with settlement concentrated during June and July.

Diurnal settlement was documented on plates left out overnight and during the day in late summer. All species exhibited a non-random diurnal settlement pattern. *A. nobilis* settled mostly at night while serpulids settled mostly during the day. *S. unicornis* settled only in the morning and midday. The three species of tunicate examined showed different peak settlement times; settlement of *Diplosoma* sp. A peaked in the morning, *Diplosoma* sp. B at midday, and *Botrylloides* sp. had maximum settlement in the afternoon.

Seasonal settlement times indicate that these organisms have different larval release patterns, with variation in both length and timing of reproductive period. Diurnal variation in settlement was correlated with larval type; organisms with long-lived larvae (serpulids and *A. nobilis*) settled at different times than did those with short-lived larvae (*S. unicornis* and tunicates). Further studies will examine the survival value of different settling times through experimental manipulations.

## Behavioral Interactions of the Butterflyfish *Chaetodon trifascialis* at Johnston Atoll<sup>21</sup>

DARBY IRONS<sup>22</sup>

The chevron butterflyfish *Chaetodon trifascialis* is found on coral reefs at Johnston Atoll and throughout the western Indo-Pacific. This species is a solitary, territorial coral feeder strongly associated with *Acropora* spp. corals. This study examines the behavioral interactions between *C. trifascialis* and both conspecifics and heterospecifics. Data were recorded for 33 individuals at three habitats in the lagoon. Habitat 1 was

an *Acropora*-rich area. Habitat 2 was an *Acropora-Montipora* spp. mixed area. Habitat 3 was a *Montipora*-rich area. Interaction data for each individual were collected during 10 consecutive 5-min periods. The number of interactions with each conspecific or heterospecific individual was recorded. There were two categories of interactions: chasing and visiting. In examining the behavioral interactions of *C. trifascialis*, the following questions were asked: Do the interactions differ between conspecifics and heterospecifics? Do the interactions differ for males and females? Do the interaction rates differ between the habitats? Conspecifics

<sup>21</sup>This research was funded by the U.S. Army, research contract no. DACA83-84-C-0019 to James D. Parrish.

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were involved in chasing and visiting behavior. Heterospecifics were only involved in chasing behavior by the resident *C. trifascialis*. Most interactions for *C. trifascialis* females occurred with a particular neighboring conspecific male. Males visited from one to three females, but not other males. Females rarely interacted with each other. Both males and females interacted with juve-

niles within their territories. The rate of interactions varied with the habitat and feeding rate. Occasionally adults aggregated in groups of up to 20 individuals that swam together in the area for up to 10 min and then returned to their individual territories. Because of the behavioral interactions observed, it is concluded that *C. trifascialis* is socially active despite its solitary territoriality.

## Formation of Subtidal Marine Stromatolites in High Energy Environment

ANTHONY T. JONES<sup>23</sup>

Formation processes of modern subtidal stromatolites may be used in the interpretation of the ancient marine environment of the Precambrian. Ancient stromatolites were major reef builders in the Precambrian through the Devonian, a span of over  $2 \times 10^9$  years. Extensive field observations and measurements were made over a two-year period on a subtidal stromatolite field in the Bahamas. A proposed model explains the occurrence of relatively large modern stromatolites in a high energy, normal salinity, subtidal environment. The modern stromatolite environment of the Bahamas is different from that of the stromatolites from intertidal hypersaline back bays of Shark Bay, Western Australia, which has been used to interpret paleoenvironments. For the Bahamian en-

vironment, necessary requirements for the development of columnar stromatolites are abundant sediment, swift tidal currents, near platform edge water super-saturated in calcium carbonate, frequent burial, and geologically rapid cementation. The Bahamian environment is more dynamic than that of Shark Bay in that tidal currents are of the order of 100 cm/sec in the Bahamas and non-existent in Shark Bay. The current assumption in literature is that stromatolites in the Precambrian may have developed in a paleoenvironment similar to the present environment of Shark Bay. Studies of the stromatolites in the Bahamas, however, suggest that a much more dynamic marine environment than currently considered may have existed in the stromatolite fields of the Precambrian.

## Chemosensory Recognition of Conspecifics by Striped Bass Juveniles<sup>24</sup>

MATTHEW W. KELLEY<sup>25</sup>

Striped bass (*Morone saxatilis*) juveniles develop within estuarine systems, partic-

ularly the Hudson River and Chesapeake Bay. Juveniles actively form schools during the day but disperse at night when they feed inshore. Schooling has been demonstrated to be socially beneficial. Efficiency of prey location, predator avoidance, and swimming hydrodynamics have all been shown to increase within a school. Therefore, once schools are formed, any individual not be-

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<sup>24</sup>This research was funded by the Graduate School of Oceanography, University of Rhode Island.

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longing to a school is at a comparative disadvantage. Striped bass juveniles apparently reform schools each morning. If individuals are to locate a school, a mechanism for identification of conspecifics should exist. Juveniles were tested using a Y-maze to determine if discrimination and attraction to conspecifics could be achieved exclusively through chemical cues.

Striped bass juveniles are strongly attracted to familiar siblings and to unfamiliar, un-

related juveniles. Juveniles can distinguish between familiar siblings and unfamiliar, unrelated juveniles (preferring familiar siblings), but cannot distinguish unfamiliar siblings from unfamiliar, unrelated juveniles. Recognition of familiar siblings may be used as a quick, uncomplicated mechanism for reforming similar schools over time, while a species-specific response may act as a safety valve for lost or disoriented individuals.

### Effects of Soil Phosphorous Supply Characteristics and the Vesicular-Arbuscular Mycorrhizal Fungus *Glomus fasciculatus* on the Growth of *Leucaena leucocephala* (Lam.) de Wit<sup>26</sup>

JOHN K. KLEINJANS<sup>27</sup>

*Leucaena leucocephala* (koa haole) plants with root systems infected by the endosymbiotic fungus *Glomus fasciculatum* do not require as high soil solution P concentrations (I = intensity) or total available P (Q = quantity) in order to achieve maximum growth as do plants with non-mycorrhizal roots. It is proposed that inoculation of soils with appropriate mycorrhizal species will increase plant growth and decrease the critical I and Q even in soils possessing an indigenous population of mycorrhizae. To investigate this theory, *L. leucocephala* plants were

grown with and without mycorrhizal inoculation in two soils, both nonsterilized and sterilized by fumigation with methyl bromide, and fertilized to three levels of I. Significant differences in growth and plant internal P concentration were recorded as early as 10 days after planting and continued through the end of the experiment at 66 days after planting. All treatment variables showed significant effects on plant growth measures. Inoculation of field soils with mycorrhizae may produce growth enhancement and decrease need for fertilizer inputs.

### The Near-Reef Distribution of Coral Reef Fish Larvae

DONALD R. KOBAYASHI<sup>28</sup>

Previous research on coral reef fish larvae has typically consisted of surface tows in

open water away from coral reefs or in oceanic water offshore. One area that has been consistently overlooked is the water column immediately adjacent to coral reefs (i.e., 1–2 m away). The purpose of this study was to evaluate the fine scale distribution of coral reef fish larvae near coral reefs. Paired surface-to-bottom samples, one near-reef and one off-reef, were gathered with a 1 m diameter .333  $\mu\text{m}$  drop net at two sites in

<sup>26</sup>The research described was performed under a 406 grant from the United States Department of Agriculture.

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Kaneohe Bay every 2–3 days for two months in the spring of 1987. Significantly higher densities of reef fish larvae were encountered immediately adjacent to reefs, with some values approaching 10 larvae per cubic meter. This difference cannot be attributed to fortuitous samplings of new hatchlings; most larvae captured near-reef are well developed and exceed the known hatching size for commonly encountered reef fish. Near-settlement larvae of *Psilogobius mainlandi* (the most common post-flexion larvae) were 20–50 times more abundant at near-reef stations than at control stations approximately 100 m away. This suggests that *Psilo-*

*gobius* larvae, whether dispersed immediately after hatching or not, can become accumulated near a reef before settlement occurs. In addition to spatial variability, there appears to be a lunar pattern of abundance in reef fish larval densities.

The results suggest that the near-reef areas must not be overlooked for a complete knowledge of reef fish larval distribution and abundance. Furthermore, assumptions of passive dispersal for pelagic larvae may have to be revised since there appear to be considerable interspecific differences in the retention/reef-seeking abilities of reef fish larvae.

## The Ecology of an Almost Anchialine Shrimp, *Parhippolyte uveae* in Hawaii

COLIN J. LAU<sup>29</sup>

The caridean shrimp, *Parhippolyte uveae*, is primarily troglomorphic in the Hawaiian Islands in contrast to populations from other Indo-Pacific localities including Fiji, the Philippines, the Moluccas, the Marshall Islands, Aldabra, and Funafuti Atoll. Although possessing many of the features of hypogeal shrimps, *P. uveae* occurs as a component of a transitional fauna between the euhaline and mixohaline hypogeal habitat. A study of feeding habits indicated habitat preference, activity pattern, and feeding strategy of the shrimp. Observations in the field and in the laboratory shrimp from the north shore of Oahu and the Kona Coast of Hawaii indicate that *P. uveae*, a facultative cave-dweller (troglophile), is present in submerged marine caves with freshwater seepage. Unlike specimens in the Philippines and Aldabra, which emerged from hypogeal sources during the daytime or at high tide to feed in anchialine pools, Hawaiian populations were observed in caves near areas of freshwater seepage

during the day and moving over the surfaces of hard substrates near the cave entrances at night. Activity patterns for Hawaiian populations as well as observations taken in other Indo-Pacific localities can be explained as avoidance of predators. Siliceous sponge spicules of an unidentified demosponge and detritus comprised over 80% by volume of cardiac stomach contents. Isopods and copepods in the diet indicate an opportunistic feeding mode with a potential cleaning symbiosis with fishes. By comparison, shrimp from Aldabra Atoll and the Philippines were observed to feed on snails and algae in the anchialine system. Colonization of anchialine pools by *P. uveae* may have already occurred at Cape Kinau, Maui, but no evidence of such an event has been observed in other Hawaiian anchialine habitats. Instead, *P. uveae* has been found in marine caves and tubes with apparent connections to the hypogeal habitat. Barriers to colonization of anchialine pools may include physical blockage as well as such physico-chemical factors as dissolved oxygen.

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## Sexual Dimorphism, Dichromatism, and Protogynous Hermaphroditism in the Pomacanthid Angelfish, *Centropyge potteri*<sup>30</sup>

MARVIN LUTNESKY<sup>31</sup>

Potter's angelfish, *Centropyge potteri*, is endemic to the waters around the Hawaiian Islands and Johnston Atoll. It occurs in social units of one male with one to eight females. It is potentially a good subject for studying harem formation and sex change theory. First, however, it must be shown that the sex of an individual can be accurately identified in the field, and that *C. potteri* is a protogynous hermaphrodite as are many other angelfishes in the genus *Centropyge*. The purpose of this study is (1) to demonstrate that *C. potteri* is sexually dimorphic and dichromatic, (2) to demonstrate that the dichromatism can be used as a diagnostic feature to accurately identify sex in the field, and (3) to show by histological evidence that *C. potteri* is protogynous.

Fifty-two *C. potteri* were caught by spear or handnet at Portlock, Oahu. Standard length, head length, preopercular spine length, and color pattern dimensions were measured. Males were significantly longer than females and had longer preopercular spines relative to head length. It appeared that the relative size of the spine grew with body length, that is, males had larger spines because they were larger. A color index of

the amount of dark blue pigment on the side of the body was developed. With the use of this index, it was revealed that dark blue pigment covered a greater proportion of the sides of males than females. Color index score was related to sex, not simply body size. Based on color pattern, individuals were sexed in the field and out of water in isolation. The same individuals were later sexed histologically. There were no significant differences in the frequencies of the sexes identified between the three methods.

Histological evidence of protogynous hermaphroditism in *C. potteri* included lamellar organization in testes apparently resulting from lamellar organization in ovaries; testes with a membrane-bound central cavity; testes with atretic bodies in stages 2 and 3 of oocyte atresia; and testes with sperm sinuses in the gonadal wall.

Given the results of this study, it was concluded that *C. potteri* is a protogynous hermaphrodite that is sexually dimorphic and dichromatic, and can be accurately sexed in the field. This study opens a pathway for future studies on harem formation and sex change theory using *C. potteri* as a test species.

## Soil Solution Phosphorus Status and Mycorrhizal Inoculation Effect in *Leucaena leucocephala*

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Phosphorus deficiency is probably a major limiting factor to the growth of legumes in

tropical, highly weathered soils, where the phosphorus-fixing capacities of the soils are high. Research undertaken over the past three decades has established that Vesicular-arbuscular mycorrhizal (VAM) fungi can improve phosphorus (P) uptake and plant growth in soils having low fertility. When compared to surface soils, subsoils are low in

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available P concentration and propagule density of VAM fungi, and have higher P-fixing capacity. Inoculation with VAM fungi is particularly important in situations where topsoils are lost due to erosion. The objective of the investigation was to study the influence of inoculation of a subsurface soil of an Oxisol with *Glomus fasciculatum* (VAM fungus) at various levels of soil solution P concentration on growth and nutrient uptake of *L. leucocephala*, an important tropical tree legume.

Phosphorus sorption isotherm was used to establish P concentrations in the soil solution ranging from 0.002–2.56 mg/L. The influence of inoculation of soil with *G. fasciculatum* on nutrient uptake and growth of *L. leucocephala* was evaluated in a pot experiment. The development of VAM activity was monitored by determining the P concentration of the second sub-leaflet of the youngest fully expanded *Leucaena* leaf at regular intervals. Plants were allowed to grow for 50 days, after which measurements were made on shoot and root dry matter, phosphorus, zinc and copper contents of shoot and root, and VAM colonization levels.

Sub-leaflets of inoculated *Leucaena* had significantly higher concentration of P than those of uninoculated *Leucaena* starting from 22 days up to 38 days after planting at P levels ranging from 0.002–0.08 mg/L. The differences between inoculated and uninoculated *Leucaena* in P concentration of sub-leaflets decreased as the level of P in the soil

solution increased. The level of mycorrhizal colonization increased with increase in the concentration of P in the soil solution up to 0.32 mg/L. Although higher P levels depressed VAM colonization, the level of colonization never declined below 60%. Dry weight of shoot and root was significantly increased by mycorrhizal inoculation with increases in P concentration in the soil solution up to 0.32 and 0.16 mg/L, respectively. Root dry weight was significantly reduced at higher P levels (1.28 and 2.58 mg/L). Shoots and roots of inoculated *Leucaena* had higher phosphorus, zinc, and copper contents than those of uninoculated *Leucaena* at all levels of soil solution P tested. Inoculated plants removed higher quantities of P from soil than uninoculated plants at all levels of P in the soil solution. Mycorrhizal inoculation effect decreased as the level of P in the soil solution increased.

The results of this investigation indicate that inoculation of subsurface soil with *G. fasciculatum* improves uptake of phosphorus, zinc, and copper, and growth of *Leucaena* when P concentration in the soil solution is lower than 0.32 mg/L. The concentration of P in the soil solution required by uninoculated *Leucaena* for maximum yield was 16 times higher than that required by inoculated *Leucaena*. These results illustrate the significance of optimizing soil solution phosphorus for maximizing the benefits of the VAM symbiosis.

## The Influence of Larval Duration and Larval Size on the Post-Larval Growth and Reproduction of *Phestilla sibogae*<sup>33</sup>

STEPHEN E. MILLER<sup>34</sup>

Larval period has an important influence on the distribution and abundance of benthic

marine invertebrate populations. However, empirical studies demonstrating the effects of larval duration and larval size on post-larval life-histories are lacking. Size and age at settlement are greatly influenced by larval growth and duration. If post-larval life history characters are size or age dependent

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then larval growth and duration are important in determining the timing and robustness of these characters.

Age and size components of planktotrophic and lecithotrophic larval development are discussed with regard to precompetent and competent larval periods, settlement, and post-larval growth and reproduction. Variation in the timing and length of the precompetent and competent larval periods may greatly alter the pattern of post-larval development. However, if metamorphic competence represents a temporal as well as a morphological hiatus in the developmental program, then extended larval periods should

not alter the timing and length of post-larval life history stages.

Data from the aeolid nudibranch *Phestilla sibogae* demonstrate the effects of the larval period on post-larval life history. Larvae are raised under fed and starved culture conditions for up to four weeks. Weekly subsamples are metamorphosed onto the coral *Porites compressa* and the subsequent juvenile and adult life-span are followed. Preliminary results indicate that variation in the length of larval life and larval nutrition can significantly alter post-larval development and reproduction.

## Auto-Amputation in Diamondback Moths: A New Form of Insecticide Resistance?<sup>35</sup>

AUBREY MOORE<sup>36</sup>

The rapid evolution of resistance to insecticides in more than 450 arthropod species demonstrates the power of natural selection and threatens agriculture and human health worldwide. Most insecticide resistance research has concentrated on physiological adaptations such as enhanced enzymatic detoxification, altered target sites, and reduced insecticide penetration. Behavioral resistance to insecticides has yet to be thoroughly studied. In particular, little is known about behavioral responses of adult Lepidoptera to insecticides, because insecticidal control is usually aimed at damaging the larval stages of these insects.

We discovered that adults of the diamondback moth, *Plutella xylostella*, a major pest of crucifers in more than 80 countries, drop one or more of their legs after tarsal contact with residues of insecticides. The purpose

of the research was to characterize the leg-drop response in the following terms: dose-response curve, time course, anatomical detail, effects on absorption of insecticide, and effects on fitness-related traits. To achieve our objectives, we performed a series of bioassays in which moths were dosed by enclosing them in glass tubes which had been internally coated with known amounts of fenvalerate, a pyrethroid insecticide.

At a dosage producing 10% mortality, the proportion of moths which dropped legs was 30% within 0.5 hr after treatment and 60% within 24 hr. Most moths lost only their metathoracic legs, which were severed at the joint between the trochanter and the femur. Scanning electron microscopy revealed that both sides of the break were smooth, with no indication of torn tissues. Tracer studies with radio-labeled insecticide showed that fenvalerate was 10 times more concentrated in dropped legs than in the body. Moths which drop one or more legs were significantly more likely to overcome insecticide-induced paralysis.

We conclude that some moths reduce the

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amount of toxin reaching their body by dropping legs, thereby increasing their fitness. To the best of our knowledge, this is the first report of auto-amputation by an insect

in response to toxins. Selection for individuals which drop legs after tarsal contact with insecticide residues could lead to a new form of insecticide resistance.

### **Biology of the Striped Catfishes, *Pseudoplatystoma fasciatum* and *P. tigrinum*, in the Apure River Drainage, Venezuela<sup>37</sup>**

STEWART B. REID<sup>38</sup>

Two large pimelodid catfishes, *Pseudoplatystoma fasciatum* and *P. tigrinum*, are sympatric in the Apure River drainage (Orinoco basin) and are very important in commercial freshwater fisheries. Previously unknown, the life histories of the two species show definite ecological separation, while being generally representative of many fishes in the Orinoco basin.

*P. fasciatum* reaches 130 cm SL; *P. tigrinum* is larger, reaching 175 cm SL. Males grow to about 80% of the females' size. Both species mature at near 40 cm SL in their second year.

*P. fasciatum* begins to breed at the end of the dry season (March), while most *P. tigri-*

*num* begin in April. Eggs are small and are dispersed by rising floodwaters into the grassy river margins, a nursery habitat for many local fishes. Young first appear in May.

Both are opportunistic piscivores, but do eat some shrimp. *P. fasciatum* is more diurnal and prefers habitat with cover, while *P. tigrinum* occurs more frequently in open lagoons and on beaches.

A portion of each population migrates seasonally in response to the reproductive migrations of prey (e.g., *Prochilodus mariae*), moving upriver at the end of the wet season and returning at the end of the dry season.

### **Intestinal Basolateral Membrane Vesicle Transport of Amino Acids in the Eel, *Anguilla anguilla*<sup>39</sup>**

STEPHAN J. RESHKIN<sup>40</sup>

Intestinal epithelial cells are asymmetrical and possess two types of plasma membrane differentiated into brush border and basolateral. Research on brush border amino acid

transport in mammals is quite extensive, and complex sodium independent and dependent transport systems have been characterized for a wide range of amino acids, which seem to be conserved among various mammalian groups. The basolateral membrane has been much more difficult to study because of its association with muscle and connective tissue, and consequently there has been relatively little work done on the amino acid transport systems from this side of the cell. The research that has been done suggests much more variability in the types of systems

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used by different mammalian species, such that profound dissimilarities have been described for the same amino acid by different workers. There has been no single study that examines the transport processes of a range of different classes of amino acids in the same basolateral preparation that would result in a coherent data set from which it would be possible to extract trends. Additionally, there has not been any of this type of work conducted with fish, the largest class of vertebrates. Therefore, a survey of the ability of intestinal epithelial basolateral vesicles (BLMV) of the eel, *Anguilla anguilla*, to transport amino acids was conducted using radioactive tracer and membrane potential techniques.

Eels were killed by decapitation, and intestinal epithelial cells were released by incubation of the slit intestine in a Na-citrate buffer. The BLMV were isolated by a differential-gradient centrifugation technique. Basolateral vesicles were enriched 12-fold with re-

spect to the homogenate in the basolateral membrane marker enzyme Na/K ATPase, while marker enzymes for other cellular membranes were not enriched. The survey of six L-amino acids identified three groups with respect to transport: (1) Transport of proline and glutamate occurred by Na-dependent carrier systems and simple diffusion; (2) Alanine, lysine and phenylalanine were transported by Na-independent carriers and simple diffusion; and (3) Glycine uptake rate was very low, was not stimulated above simple diffusion by a Na gradient, and was inhibited by a K gradient. Sodium dependent glutamate uptake was not further stimulated by intravesicular K or an outwardly directed K gradient. Only proline and glutamate demonstrated the ability to depolarize the membrane potential, consistent with Na-dependent transport and suggestive of a greater than 1:1 Na:glutamate stoichiometry.

## The Accumulation of Cadmium and Its Effects on Growth of Larvae of an Hawaiian Bivalve, *Isognomon californicum*

AMY HUFFMAN RINGWOOD<sup>41</sup>

Early bivalve larvae were exposed to 2 and 20 ppb Cd for 14 days. The cultures were then subdivided: some larvae were exposed to Cd for an additional 14 days; the remainder were ongrown in clean seawater. Periodically, samples were taken for measurement of growth and Cd concentrations. Growth in the presence of Cd is characterized by three phases: an initial period of depressed growth, a period of partial recovery, and stabilization of growth at a level significantly lower than

the controls. Cadmium accumulates during the initial phase of depressed growth. After 7 days of exposure, during the period of partial recovery, there is a dramatic increase in Cd accumulation. These results are consistent with the hypothesis that bivalve larvae are capable of concentrating and sequestering Cd, probably through the production of metallothioneins, which are effective after 7 days of exposure. In those larvae that were ongrown in clean seawater, a substantial loss of Cd was observed, but there was no improvement in growth. This indicates that the adverse effects of Cd exposures are irreversible.

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## Evolutionary Game Theory Applied to Interspecific Aggression Among Corals: Are Corals Really Bullies?

SANDRA L. ROMANO<sup>42</sup>

Sessile marine organisms such as scleractinian corals have evolved aggressive strategies for living in an environment that is often limited in space. Direct spatial competition occurs primarily by means of extracoelenteric digestion. The outcome of an interaction is determined by the relative positions of the two interacting species in the aggressive hierarchy of the assemblage. Little attention has been given to the evolution of such complex interactions between relatively simple organisms.

Interspecific interactions between corals can be considered as contests for space between two colonies. The cost and benefits of the contest are measured in terms of fitness. A coral coming in contact with another coral may have one of three reactions: to always attack the other coral, to never attack the other coral, or to attack the other coral only if it is of lower rank in the aggressive hierarchy. This last reaction can be defined as the strategy of a Bully. In a population where the cost of a contest—the cost of fighting—is high but less than the benefits gained from winning a contest, individuals having the strategy of a Bully will have a higher fitness than individuals that have either of the other two strategies. A population composed of Bullies would therefore be uninvadable by any mutants having either of the two other strategies.

The hierarchy of aggression among the five most common reef flat species of coral in

Hawaii was determined by observing interspecific interactions in Kaneohe Bay, Oahu. Colonies of *Pocillopora damicornis*, *Porites compressa*, *Cyphastrea ocellina*, *Montipora verrucosa*, and *Fungia scutaria* were paired on the reef flat of Coconut Island in Kaneohe Bay. Colonies were placed within soft tissue contact and held together with rubber bands. One hundred fourteen interacting pairs were monitored over periods of 10–14 days during July and August 1986.

*F. scutaria* was found to be the dominant aggressor followed by *C. ocellina*, *P. compressa* and *M. verrucosa*, and *P. damicornis*. Of the interactions, 87% resulted in unilateral damage by the species of higher rank to the species of lower rank. Similar results have been obtained in other studies of interspecific aggression among corals. Out of 817 published interactions, 85% resulted in clear dominance of one coral over the other.

A coral does have the ability to identify the aggressive rank of another colony with which it comes in contact. This ability is used so as to fight only when there is a very high probability of winning. These observations seem to support the hypothesis that corals have evolved the strategy of a Bully for interspecific interactions. Whether this is an evolutionarily stable strategy depends on the relative costs and benefits of a contest. Research is currently under way to evaluate these relative costs and benefits.

## Differential Uptake and Metabolism of Retinol and Retinoic Acid in 10T1/2 Cells<sup>43</sup>

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<sup>43</sup> This study was supported by Grant CA 39947 from the National Cancer Institute.

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Vitamin A (retinol) and its analogs have been shown to be potent inhibitors of carcinogenesis in both *in vivo* animal models and *in vitro* cell cultures. In most systems, the

acid form (retinoic acid) is many times more potent than retinol. However, in the mouse embryo fibroblast C3H/10T1/2 cell line, retinoic acid does not inhibit carcinogen-induced transformation, except at toxic levels, while retinol effectively inhibits transformation at nontoxic doses. This cell line closely reflects other *in vivo* events; and other vitamin A analogs, active *in vivo*, are also inhibitory in the 10T1/2 cell line. In an attempt to explain this anomaly, I have studied the differential metabolism of retinol and retinoic acid by 10T1/2 cells and their initiated and transformed derivatives.

Confluent cultures were treated with  $10^{-6}$ M retinoic acid or retinol. Cells and medium aliquots were collected at various times post-treatment and extracted with n-butanol: acetonitrile (1:1). The extracts were analyzed by reverse phase (Spherisorb ODS-2, C-18 column) high performance liquid chromatography (HPLC). A medium treated with retinol or retinoic acid and incubated in the absence of cells was also collected and extracted to determine degradation due to incubation and extraction procedures.

While retinoic acid was rapidly depleted from the culture medium of 10T1/2 and initiated 10T1/2 cells by 48 hr, retinol disappeared

from the culture medium no faster than from cell-free medium (half-life, 34 hr). Lack of metabolism did not correlate with lack of uptake into cells, as both retinoic acid and retinol were rapidly concentrated by cells (23- and 500-fold over medium levels, respectively, after 5 hr). After 96 hr, however, retinoic acid levels were 4 pmol/ $10^6$  cells, whereas retinol maintained a level of 200 pmol/ $10^6$  cells. Retinoic acid induced its own metabolism, the rate and extent of which varied markedly among four transformed lines tested, in contrast, retinol was metabolized at similar low rates in all cell lines.

In the standard 10T1/2 transformation assay, the cells are treated with potential inhibitors or promoters of transformation with weekly medium changes, after the initial carcinogen treatment, and are scored after 5–6 weeks. Thus, this study suggests that the inability of retinoic acid to inhibit transformation in the 10T1/2 cell system may be due to its rapid metabolism and clearance from the medium. On the other hand, the high cellular uptake and stability of retinol in these cells, much of it presumably in the membranes, could be an important factor in the mechanism by which retinol inhibits transformation.

## Effect of Nutrient Enrichment on the Zooxanthellae of a Reef Coral, *Pocillopora damicornis*<sup>45</sup>

ANOND SNIDVONGS<sup>46</sup>

Coral reefs are thought by some workers to be adapted to environments where nutrient levels are limited. Symbioses between unicellular algae, called zooxanthellae, and many coelenterates, including reef-building corals, are hypothesized to be an adaptation to the problem of nutrient shortage. This

study investigates the effect of nutrient levels on symbiotic algae.

*Pocillopora damicornis*, a symbiotic coral, was kept in four controlled nutrient regimes for 8 weeks.

1. Control: Ambient unfiltered Kaneohe Bay seawater normally contains approximately 1.1  $\mu$ m of total dissolved inorganic nitrogen and approximately 0.3  $\mu$ m of dissolved reactive phosphorus.

2. Nitrogen addition as ammonium

<sup>45</sup> Parts of this study were supported by the East-West Center.

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chloride to approximately  $15\ \mu\text{m}$  above ambient level.

3. Phosphorus addition as potassium phosphate (monobasic) to approximately  $1\ \mu\text{m}$  above ambient.

4. Both nitrogen and phosphorus addition to the levels of 2 and 3.

The nutrients were continuously added during the course of the experiment. Pre- and post-experiment levels of total carbon (C), total nitrogen (N), and total reactive phosphorus (P) were determined for zooxanthellae isolated from host tissue.

In the control treatment with ambient nutrient levels, atomic N : C and P : C ratios of symbiotic zooxanthellae were well below Redfield ratio, suggesting nutrient limitation. The deviation of the P : C ratio was about two times more than the N : C ratio. Nitrogen enrichment resulted in an increased

cellular nitrogen content indicating "luxury" nitrogen consumption. When only phosphorus was added, both C per cell and P per cell decreased, suggesting the possibility of smaller cell, and thus, higher cell division rate.

Interpretation of the result from the phosphorus enriched experiments remains obscure. Under these experimental conditions, zooxanthellae did not respond to elevated phosphorus by increasing cellular phosphorus content and P : C ratio, even when the N : P ratio of the water was about 1 : 1. This is possibly because the *in situ* availability of phosphorus was not enhanced by the same mechanism as nitrogen. The coral host may play a role in controlling the nutrient environment, especially for phosphorus, for its endosymbiotic algae.

### Response to Supplemental Feeding by a Non-Breeding Wild Population of Maomao, *Abudefduf abdominalis* (Pisces: Pomacentridae)

FRANK G. STANTON<sup>47</sup>

Commercial fish food was provided to colonies of maomao, testing the hypothesis that supplemental feeding would induce spawning in a non-breeding colony. The study was conducted at the Hawaii Institute of Marine Biology on Moku O Loe (Coconut Island) in Kaneohe Bay, Oahu. Daily spawning activities were recorded at experimental and control sites 6 weeks prior to, during, and 6 weeks after the treatment. Samples of liver, gut, gonad, and muscle tissues from fish collected before and after the treatment indi-

cated that the experimental group in the non-breeding colony accumulated more lipids than a control population that was not fed supplemental fish food. Spawning in the non-breeding colony was not induced during the six-week period of supplemental feeding, however when spawning began three weeks after the feeding ended, the experimental colony had more clutches than the control colony. A breeding colony was also fed additional food and responded immediately with an increase in spawning.

### Multiple Parentage in the Permanently Monogamous Common Myna, *Acridotheres tristis*<sup>48</sup>

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<sup>48</sup> This study was supported by the National Science Foundation.

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Most bird species are considered to be monogamous, based on associations between one male and one female and shared parental

duties toward one group of young. However, electrophoretic studies of blood proteins of apparently monogamous parents and associated offspring reveal that one or both parents may be rearing the offspring of other adults. The observation that some nestlings are not related to the male of the pair indicates that the female has copulated with a male other than her mate. In addition, intraspecific brood parasitism (when an extra-pair female lays eggs within the nest of a pair) may result in nestlings which are unrelated to the female. Individuals that invest in unrelated offspring do so at the expense of both current and future related offspring.

Information on the breeding biology of a banded population of the common myna, *Acridotheres tristis*, has been collected over the past two years. Mynas readily nest in artificial boxes and return to the same box, or one nearby, for subsequent clutches. They are permanently monogamous, with pair-mates remaining together both during the breeding season (December through July), and between breeding seasons. They may lay up to three successful clutches per breeding season. Parental care constitutes a considerable investment for both male and female. The female incubates more than the male; however, both male and female feed the young and defend a territory around the nest from conspecifics. Behavior suggesting extra-pair copulations or intraspecific brood parasitism has not been observed. However, throughout the year mynas attend a social sleeping roost composed of hundreds of in-

dividuals. It is presumed that the female attends the roost even during her fertile period; this is currently under investigation. It is therefore possible that extra-pair copulations occur at the roost. It is also possible that intraspecific brood parasitism occurs at the nest before the resident pair returns to the territory from the roost. Common mynas provide an interesting example of a species for which apparent permanent monogamy occurs within a social environment that potentially threatens genetic paternity and maternity.

The potential for multiple parentage was examined in the common myna. Thirty-three loci were screened for polymorphisms in myna blood using horizontal starch gel electrophoresis. Six polymorphic loci were resolved (PEPTD, EST, 6-PGD, AK, CPK, EAP). Blood was collected from individuals within nine myna families. The occurrence of multiple parentage was determined by matching the blood enzyme polymorphisms of the adults and chicks within each family. Preliminary results examining plasma esterase indicate that intraspecific brood parasitism occurred in one of the nine families. There was no evidence for extra-pair copulations. Results from the examination of the remaining polymorphic loci using more families may lead to the detection of other cases of multiple parentage. The results of this study emphasize the way in which sociality may incur costs due to the increased risk of multiple parentage and may consequently affect the fitness of the individual.

### Short-chain Fatty Acid Transport in Intestinal, Brush Border Membrane Vesicles of the African Tilapia *Oreochromis mossambicus*<sup>50</sup>

ERIC TITUS<sup>51</sup>

Short-chain fatty acids (SCFA: acetate, propionate, and butyrate) are generally derived from anaerobic fermentation of di-

etary fiber. In herbivores and omnivores the primary source of these nutrients is usually plant polysaccharide, though there may be other sources such as mucus or unabsorbed starches. Short-chain fatty acid production has been calculated to provide significant proportions of maintenance requirements in

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some mammals, ranging from 7% in dogs to 80% in ruminants, and it has been shown to have a pronounced effect on sodium and water transport in ground fowl. Earthworms and tapeworms have been demonstrated to transport SCFA transintegumentarily, and several cellulose-ingesting insects have been observed to absorb SCFAs across the hindgut. These observations suggest the wide occurrence of SCFA use among organisms; however, surprisingly little research of intestinal transport mechanisms for this group of compounds has been undertaken.

The current study addresses the yet-unknown question of SCFA transport in a herbivorous teleost. The tilapia, *Oreochromis mossambicus*, was analyzed for SCFA content in the gut lumen by gas chromatography. Concentrations of acetate ranging from 3–12 mM were found along the full length of the intestinal tract, as were trace amounts of propionate. Characteristics of  $^3\text{H}$ -acetate transport by the intestinal brush border membrane were analyzed in isolated brush border membrane vesicles (BBMV). Vesicles were prepared using a magnesium chloride precipitation/differential centrifugation method.

Influx of  $^3\text{H}$ -acetate into BBMV occurred by apparent anion exchange and was not enhanced by inwardly-directed gradients of sodium or potassium, indicating the lack of sodium-dependent coupling for acetate transport. Competition and stimulation studies with various organic and inorganic anions revealed the likelihood of a transport system in which  $^3\text{H}$ -acetate is exchanged specifically for intracellular bicarbonate or other SCFA. Other anions tested had only limited or negligible effects on  $^3\text{H}$ -acetate uptake. Kinetic analysis over a concentration of external acetate from .05–50 mM yielded a carrier transport  $K_{\text{m}}$  of 5 mM and a  $V_{\text{max}}$  of 17 pmol/mg prot/10 sec.

This study suggests the occurrence of a specific antiport system for anions which is shared by bicarbonate and short-chain volatile fatty acids in the intestinal brush border membrane. The value for  $K_{\text{m}}$  approximates the concentration of acetate found in the intestinal lumen of the fish, further supporting the relevance of such an SCFA transport system. This mechanism may facilitate the exchange of potentially nutritive luminal anions (SCFA) for cellular anionic waste products ( $\text{HCO}_3^-$ ).

## Geochemistry of Reef Interstitial Waters

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In order to investigate biogeochemical processes inside submerged carbonate structures, water was sampled from the internal framework of a coral reef in Kaneohe Bay, Oahu. Dissolved oxygen levels were low or undetectable in the interstitial water and concentrations of dissolved methane, sulfide, and inorganic carbon were elevated. The reef interstitial water also differed from surface water in having a lower pH and higher alkalinity. Dissolved organic nitrogen and phosphorus concentrations were similar in inter-

stitial and overlying waters, but interstitial waters were enriched with inorganic nutrients. Salinity measurements indicated that groundwater seepage did not influence the composition of the reef interstitial water. The differences in chemical composition between reef surface water and reef interstitial water appear to result from microbial oxidation of organic matter through both oxic and anoxic pathways. Preliminary data suggests that, at least locally, microbial activity results in the net dissolution of carbonates.

Hydrological investigations indicate that the reef interstitial waters are advectively driven and that exchange with the overlying

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seawater occurs on a time period of several hours to a few days. The flux of nutrient-enriched water out of the reef framework

may thus represent a significant source of remineralized nutrients to reef primary producers.

## Spawning Patterns in the Hawaiian Sergeant, *Abudefduf abdominalis* (Family: Pomacentridae)<sup>53</sup>

WILLIAM A. TYLER III<sup>54</sup>

Several hypotheses are proposed to account for observed periodicity in spawning of coral reef fishes. An increase in the probability of progeny dispersal away from home reefs or escape from reef-based predators are suggested as possible factors contributing to lunar periodicity observed in many species. Maximum tidal flushing occurs at new and full moons. Some species may use environmental cues which exhibit predictable cycles to synchronize reproductive condition in the breeding population. Breeding synchrony may increase the probability of locating a receptive mate or may be a numerical response to predation.

The reproductive behavior of two populations of the Hawaiian sergeant or maomao, *Abudefduf abdominalis*, have been studied on two patch reefs in Kaneohe Bay, Oahu since June 1984. Males defend individual nests of one to six clutches from egg predators. The clutches are produced by one or more females. Nesting males frequently form aggregations of up to 30 active nests in regularly used sites on each study reef. The location and developmental stage of each clutch spawned on the two patch reefs were censused at least every five days which is the minimum development time for hatching.

From these data, estimates of the spawning data for each clutch were made by subtracting the age of each developmental stage from the sampling date. Corrections were made for temperature dependent variation in egg development rates.

Using autocorrelation and spectral analysis procedures, periods of one and approximately five days were consistently observed in the portions of continuous time series data analyzed for both populations. Crosscorrelative analysis between both populations for these same time periods also indicate a relatively strong correlation in spawning frequency. The 5-day period may correspond to the minimum developmental time for hatching in this species. Usually several clutches are spawned in a given nest over a period of one to two days which may account for the 1-day period observed in the analyses.

These results suggest that social factors may play an important role in the periodicity of spawning behavior in *A. abdominalis*. Females may synchronize spawning within a couple of days of each other during each spawning cycle. With numerous nests active simultaneously within aggregations on the reef, egg predators may be hampered by the "cumulative" defense (overlapping defended areas around nests) of nesting males within a given area, thus increasing the probability of egg survival in aggregations. Whether or not nesting aggregations increase egg survivorship and confer an advantage to the reproductive success of colonial males is the topic of current research.

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## Silver Carp (*Hypophthalmichthys molitrix*) Effects on Water Quality in Freshwater Prawn (*Macrobrachium rosenbergii*) Ponds in Hawaii<sup>55</sup>

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Macrobrachium farms in Hawaii have traditionally controlled water quality in ponds by exchanging water. Water availability is a constraint to production on some farms. Two experiments were conducted in which silver carp (*Hypophthalmichthys molitrix*) fingerlings were introduced into 0.4 ha prawn (*Macrobrachium rosenbergii*) ponds to determine whether these fish have positive effects on water quality. During the first experiment we observed a significant correlation between the average prawn harvest for each treatment and carp stocking density (four or five ponds monitored for each treatment; 0, 20, 60, and 240 fish per treatment). Fewer low AM dissolved oxygen readings were observed in ponds with 60 or more fish. Primary productivity measured in incubations via a number of different methods was higher in ponds with the low to moderate carp-stocking rates. Variability of measured quantities within treatments from the first experiment made demonstration of significant differences impossible.

A second trial was conducted with twelve replicates on each of two treatments: 0 and 30 fish per pond. Heteroscedasticity of the data required the application of nonparametric tests. The silver-carp-stocked ponds

showed levels of particulate chlorophyll a, carbon, and nitrogen significantly elevated relative to control ponds (Mann-Whitney Wilcoxon rank test,  $P < .0001$ ). Frequency of water flow from the two-inch inlet pipes was marginally smaller in fish ponds than in controls ( $P < .1$ ). The concentrations of dissolved inorganic nitrogen and the ratio of dissolved inorganic nitrogen to soluble reactive phosphorus were lower in fish ponds than in controls (ANOVA  $P < .05$  and  $.01$  respectively).

It is not reasonable to accept that the low densities of silver carp we stocked in these ponds could have daily grazed any substantial fraction of the phytoplankton standing stock. We have speculated that two mechanisms might be responsible for the changes observed in the ponds with fish. By consuming small herbivorous zooplankton along with larger phytoplankton, the silver carp may reduce competition and grazing pressure on phytoplankton too small for them to retain in their gill rakers. Secondly, the swimming activity of the large silver carp may resuspend sediments and thereby enhance rates of inorganic nutrient remineralization. Experiments to test these hypotheses are being planned.

Very low stocking densities of silver carp grow rapidly in prawn ponds and cause increases in the phytoplankton biomass. Where a market for these fish exists and the hazards associated with manual net-harvesting ponds containing silver carp can be avoided, it seems advantageous to polyculture silver carp with prawns.

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## Temperature and Photoperiod Effects on Ovarian Maturation in the Chinese Catfish, *Clarias fuscus*<sup>57</sup>

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In many temperate and subtropical teleost fishes, seasonal changes in photoperiod and temperature trigger gonadal development. The Chinese catfish, *Clarias fuscus*, found in China, Taiwan, and Hawaii, typically spawns during the summer months of June through September. Ovarian maturation in this species may be stimulated by the separate or combined effects of the increased photoperiod and temperature characteristic of summer.

To test this hypothesis, 100–150 g, one-year-old catfish obtained from a prawn pond near Kohala, Hawaii, were subjected to different photoperiod and temperature regimes over a one-year period. Treatments consisted of four temperatures: 20, 25, 30, and 35°C; and five photoperiods within each temperature treatment: 10 hr light:14 hr dark (winter), 14 hr light:10 hr dark (summer), constant light, darkness, and ambient light. Each 1 m-diameter, 30 cm-deep treat-

ment tank was stocked with 10 females and 10 males. Experiments commenced during the November to December post-spawning period. Oocyte development was monitored by catheterization, and degree of abdominal distention noted. When females within a given photo-temperature treatment developed mature ovaries (mean oocyte size greater than 1.5 mm, distended abdomens), they were induced to ovulate using a 4 IU injection of HCG. Eggs were then fertilized with milt obtained from the males within a given treatment. Hatch rates and fecundities were recorded.

Temperature had the strongest effect on ovarian maturation in terms of oocyte growth and fecundity. At 25°C under constant or long photoperiod stimulus, females developed mature ovaries and were induced to spawn successfully every 1.5 to 3 months. At 30°C, regardless of photoperiod, ovaries matured, but over 3 month or longer intervals. Maturation was retarded at 20, 25 (short photoperiods), and 35°C. Fish held for 3 months in 20° and transferred to 30°C, without any change in photoperiod, developed mature ovaries within 1.5 to 3 months.

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